

Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease

Article

Published Version

Open Access (Wiley Online Open)

Soldati, C., Bithell, A., Conforti, P., Cattaneo, E. and Buckley, N. J. (2011) Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease. *Journal of Neurochemistry*, 116 (3). pp. 415-425. ISSN 0022-3042 doi: <https://doi.org/10.1111/j.1471-4159.2010.07122.x> Available at <https://centaur.reading.ac.uk/34707/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1111/j.1471-4159.2010.07122.x>

Publisher: Wiley-Blackwell

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Rescue of gene expression by modified REST decoy oligonucleotides in a cellular model of Huntington's disease

Chiara Soldati,* Angela Bithell,* Paola Conforti,† Elena Cattaneo† and Noel J. Buckley*

*Department of Neuroscience and Centre for the Cellular Basis of Behaviour, Institute of Psychiatry, King's College London, The James Black Centre, London, UK

†Department of Pharmacological Sciences and Centre for Stem Cell Research, University of Milano, Milan, Italy

Abstract

Transcriptional dysfunction is a prominent hallmark of Huntington's disease (HD). Several transcription factors have been implicated in the aetiology of HD progression and one of the most prominent is repressor element 1 (RE1) silencing transcription factor (REST). REST is a global repressor of neuronal gene expression and in the presence of mutant Huntingtin increased nuclear REST levels lead to elevated RE1 occupancy and a concomitant increase in target gene repression, including brain-derived neurotrophic factor. It is of great interest to devise strategies to reverse transcriptional dysregulation caused by increased nuclear REST and determine the consequences in HD. Thus far, such strategies have involved RNAi or mutant REST constructs. Decoys are double-stranded oligodeoxynucleotides corresponding to the

DNA-binding element of a transcription factor and act to sequester it, thereby abrogating its transcriptional activity. Here, we report the use of a novel decoy strategy to rescue REST target gene expression in a cellular model of HD. We show that delivery of the decoy in cells expressing mutant Huntingtin leads to its specific interaction with REST, a reduction in REST occupancy of RE1s and rescue of target gene expression, including Bdnf. These data point to an alternative strategy for rebalancing the transcriptional dysregulation in HD.

Keywords: brain-derived neurotrophic factor, decoy oligonucleotides, histone modification, Huntington's disease, repressor element 1 silencing transcription factor, transcriptional dysregulation.

J. Neurochem. (2011) **116**, 415–425.

Huntington's disease (HD) is an autosomal-dominant disorder characterised by neurodegeneration that leads to irrepressible motor dysfunction, cognitive decline and psychiatric disturbances, progressive dementia and death approximately 20 years after disease appearance (Zuccato *et al.* 2010). HD results from an aberrantly expanded repetition of a CAG nucleotide triplet in the huntingtin (Htt) gene that produces a polyglutamine extension at the N-terminus of the Htt protein (Huntington's Disease Collaborative Research Group 1993; Bates 2005). Although the abnormal protein is ubiquitously expressed, cell degeneration occurs mainly in the brain. Over the course of HD, the degeneration of GABA-releasing spiny projection neurons in the striatum is predominant; however, loss of neurons in other brain regions, such as cerebral cortex has also been reported (Zuccato *et al.* 2010). Htt is a large multi-domain protein, whose function is still not fully understood but is thought to have multiple distinct cellular roles, including intracellular transport, synaptic function, anti-apoptotic activity and transcriptional regulation (Cattaneo *et al.*

2005). However, much attention has focussed on the transcriptional dysregulation that occurs in the presence of mutant Htt (muHtt). Both wild-type and muHtt can interact with a number of transcription factors such as cAMP-response element binding protein-binding protein (Steffan *et al.* 2000; Nucifora *et al.* 2001), TATA-binding protein

Received August 31, 2010; revised manuscript received November 11, 2010; accepted November 12, 2010.

Address correspondence and reprint requests to Prof. Noel J. Buckley, Department of Neuroscience and Centre for the Cellular Basis of Behaviour, Institute of Psychiatry, King's College London, The James Black Centre, 125 Coldharbour Lane, London SE5 9NU, UK. E-mail: noel.buckley@kcl.ac.uk

Abbreviations used: Bdnf, brain-derived neurotrophic factor; ChIP, chromatin immunoprecipitation; Chrm4, M4 muscarinic receptor gene; DN, dominant-negative; EMSA, electrophoretic mobility shift assay; H3K9ac, histone H3 acetyl-lysine 9; HD, Huntington's disease; HDAC, histone deacetylase; Htt, Huntingtin; muHtt, mutant Huntingtin; NSCs, neural stem cells; ODN, double-stranded oligonucleotide decoy; qPCR, quantitative PCR; RE1, repressor element 1; REST, RE1 silencing transcription factor; Syn1, synapsin 1.

(Huang *et al.* 1998), Sp1 (Dunah *et al.* 2002), p53 (Steffan *et al.* 2000; Bae *et al.* 2005), transcription initiation factor TFIID 130 kDa subunit (Dunah *et al.* 2002) and TATA-binding protein (Huang *et al.* 1998) and these interactions are frequently disrupted in the presence of muHtt (Rubinsztein and Carmichael 2003). Recent work has highlighted repressor element 1 (RE1) silencing transcription factor (REST) (also known as neuron-restrictive silencing factor) as one transcription factor, whose function is disrupted in HD (Zuccato *et al.* 2003, 2007; Bithell *et al.* 2009; Johnson and Buckley 2009). REST was first identified as a regulator of neuronal genes in non-neuronal cells (Kraner *et al.* 1992; Mori *et al.* 1992; Schoenherr and Anderson 1995). Genome-wide chromatin immunoprecipitation (ChIP) and bioinformatic studies have subsequently revealed several thousand genomic REST binding loci (RE1 sites), which are linked to genes that encode non-neuronal as well as neuronal proteins (Bruce *et al.* 2004; Johnson *et al.* 2006, 2007, 2008a). Further, REST regulates target genes in both neural progenitors (Johnson *et al.* 2008a) and in mature neurons and glia (Palm *et al.* 1998; Abrajano *et al.* 2009a,b). Indeed REST dysfunction in neurons has been implicated in several neurological and neurodegenerative pathologies, including epileptic seizure (Palm *et al.* 1998; Garriga-Canut *et al.* 2006; Spencer *et al.* 2006), ischaemia (Calderone *et al.* 2003) and HD (Zuccato *et al.* 2003, 2007; Buckley *et al.* 2010).

Previous work has shown that wild-type Htt can sequester REST in the cytoplasm whereas in the presence of muHtt, this association is disrupted and leads to increased levels of nuclear REST resulting in increased REST occupancy of RE1 sites and repression of target genes (Zuccato *et al.* 2007). One key REST target gene that is repressed in the presence of muHtt is *Bdnf*. Brain-derived neurotrophic factor (BDNF) is supplied by cortical projections to the striatum where it is necessary for the maintenance and survival of medium spiny projection neurons, the most prominent neuronal population that dies during HD (Altar *et al.* 1997; Canals *et al.* 2004; Zala *et al.* 2005; Zuccato and Cattaneo 2009). Several studies have shown reduced BDNF levels in both animal and cellular models of HD. These studies indicate that a decrease in cortical BDNF occurs early in the disease process and is followed by a progressive decline, strongly implicating the role of this neurotrophin in HD pathology (Zuccato *et al.* 2001, 2007, 2008). REST occupancy of RE1 sites, including at the *Bdnf* promoter, is increased in HD models including *Hdh*^{109/109} knock-in and R6/2 mice (Zuccato *et al.* 2007). Importantly, in the case of *Hdh*^{109/109} mice, this increased occupancy precedes the development of a neuropathological phenotype (Zuccato *et al.* 2007). This suggests that dysregulation of REST target gene expression might be an early molecular event in HD and thus attenuating REST binding during early disease stages could be of therapeutic benefit.

Subsequently, we have shown that use of a dominant-negative REST construct, comprising only the eight zinc finger DNA-binding domain and lacking any co-repressor interaction domains, can rescue BDNF levels (mRNA and protein) in cellular models of HD (Zuccato *et al.* 2007). Although such mutant constructs are useful tools, they are large and require viral delivery for optimal results. An alternative strategy is deployment of decoy oligodeoxynucleotides (ODNs) that act as surrogate binding sites for transcription factors and sequester the native transcription factor from its genomic binding sites. Decoy ODNs have been used successfully to inhibit the binding and transcriptional action of several transcription factors including nuclear factor-kappaB protein, Sp1, activator protein 1, signal transducer and activator of transcription 1/3 and nuclear factor of activated T-cells 1c (Griesenbach *et al.* 2002; Chae *et al.* 2006; Stadlbauer *et al.* 2008). Furthermore, this approach has been used to successfully deliver and attenuate transcription factor function in neural cells in culture (Steiger *et al.* 2004; Meller *et al.* 2005; Yao *et al.* 2005; Aharoni-Simon *et al.* 2006), in brain slice preparations and *in vivo* (Ye and Johnson 2001; Kubo *et al.* 2003; Dash *et al.* 2005). The main limitation of unmodified ODNs is their susceptibility to degradation by nucleases prevalent in sera and cells. To rectify this problem, ODNs with modified linkages, such as phosphorothiolate and methylphosphonate, have been developed (Lee *et al.* 2003; Osako *et al.* 2007). Here, we describe the development of modified decoy ODNs to inhibit REST-mediated gene repression in a cellular model of HD and show efficient rescue of aberrant gene expression.

Methods

Hdh^{109/109} and *Hdh*^{7/7} cell culture and transfection

Hdh^{7/7} and *Hdh*^{109/109} cell lines were previously generated from the striata of wild-type and homozygous knock-in mice with each Htt allele bearing 7 or 109 CAG repeats, respectively, as described by Trettel *et al.* (2000). Both cell lines were propagated in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum at 33°C in a humidified 5% CO₂ atmosphere. Transfection with ICAfectin (Eurogentec, Southampton, UK) was performed according to manufacturer's instructions and optimal transfection efficiency (50–60%) was achieved using 0.5 µg of DNA/µL of ICAfectin. Transfections were carried out in the presence of serum and medium was replaced after 12–24 h.

Construction of phosphorothiolated RE1 decoy ODN

Single stranded oligonucleotides were synthesised by Sigma Genosys (St. Louis, MO, USA). Annealing was performed in 10× annealing buffer (100 mM Tris-HCl, pH8.0, 10 mM EDTA, 1 M NaCl) by heating to at least 5–10°C above their *T_m* and cooling slowly to 4°C over 3–4 h using a heatblock. A RE1 decoy (++D) was designed corresponding to a canonical REST-binding site (Johnson *et al.* 2006). A negative decoy control (--D) was generated using a sequence corresponding to a non-canonical RE1

that has been shown not to bind REST (Bruce *et al.* 2004). The decoy ODN sequences, including a phosphorothiolate modification on the first three nucleotides were: Positive decoy (++D): 5'-G^{pp}C^pPTPTTCAGCACCGACGACGCGCCAGC-3'; Negative decoy (--D): 5'-G^{pp}C^pPTPTCCAGCACAGTGGTCAGACCC-3'.

DNA binding and electromobility shift assay (EMSA)

For gel shift analysis, 12.5 nM of infrared (IR700) fluorescence-labelled decoy (IR-Decoy) was incubated with 0.25 µg HeLa cell nuclear extract in binding buffer (40 mM Tris-HCl, pH8, 0.4 mg/mL bovine serum albumin, 200 mM ZnCl₂, 400 mM KCl, 2 mM MgCl₂, 40% glycerol, 0.4% NP-40, Sigma, St. Louis, MO, USA) and 2 mM β-mercaptoethanol for 1 h at 4°C in the dark. For supershift experiments, 2 µL of anti-REST rabbit polyclonal (P-18X, 2 mg/mL; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to the mixture. For excess negative decoy assays, 50 nM final concentration of negative decoy (--D) was added to the mixture. Reaction mixtures were separated on a 4% non-denaturing polyacrylamide gel at 4°C in 0.5× TBE (90 mM Tris borate, 2 mM EDTA, pH8.3) at 150 V for 2 h at 4°C in the dark. The gel was pre-run at 150 V for 30 min at 4°C. Gels were scanned with an Odyssey Infrared Imaging System (Li-cor, NE, USA). The decoy ODN sequences used, including 5'-/5IRD700 modifications, were 5'-/5IRD700/TTCAGCACCGACGACGCGCC-3' and 5'-/5IRD700/GGCGTGTCCGTGGTGCTGAA-3' (Integrated DNA Technologies, Belgium).

RNA extraction and cDNA synthesis

Total RNA was extracted from *Hdh*^{109/109} and *Hdh*^{7/7} cells using Trizol (Invitrogen, Carlsbad, CA, USA) and digested with DNase I (Ambion, Austin, TX, USA). For each RNA sample, 2 µg of total RNA was reverse transcribed for 60 min at 37°C with random hexamers (Promega, Madison, WI, USA) and Moloney murine leukemia virus reverse transcriptase (Promega, WI, USA).

Real-time PCR and quantification of gene expression

Polymerase chain reactions were run with the following condition: 95°C for 3 min, then 45 cycles × (30 s 95°C, 30 s 60°C, 30 s 72°C). Real-time PCR was performed using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) with 0.5 µM primers. Expression levels were analysed according to the Pfaffl method (Pfaffl *et al.* 2002) and then normalised to levels of Gapdh. Error bars show the standard deviations obtained from two samples run in triplicate in two different assays. Primer sequences – Gapdh (Fwd: 5'-TCCGACTTCAACAGCAACTC-3'; Rev: 5'-CTTGCTCAGTGTCTTGCTG-3'); REST (Fwd: 5'-CGAACTCACACAGGAGAACG-3'; Rev: 5'-GAGGCCACATAATTGCACTG-3'); M4 muscarinic receptor gene (Chrm4) (Fwd: 5'-TCCTCACCTGGACACCCTAC-3'; Rev: 5'-ACGTAGCAGAGCCAGTAGCC-3'); Bdnf (Fwd: 5'-TCGTTCTTTCGAGTTAGCC-3'; Rev: 5'-TTGGTAACGGGCACAAAACA-3'); synapsin 1 (Syn1) (Fwd: 5'-GAGCAGATTGCCATGTCTGA-3'; Rev: 5'-CACTGCGCAGATGTCAAGTC-3').

Chromatin immunoprecipitation

Cells were fixed in 1% formaldehyde at 20–25°C for 5 min before washing in cold phosphate-buffered saline and centrifugation at

2200 g for 5 min. Cells were lysed in lysis buffer for 30 min on ice, cell nuclei pelleted and resuspended in shearing buffer before sonication in a Bioruptor (Diagenode, Belgium) to obtain an average chromatin shear size of 200–500 bp. All steps included protease inhibitor cocktail (Roche Molecular Biochemicals, Indianapolis, IN, USA). Pre-cleared chromatin (20 µg) was immunoprecipitated with 1–2 µg of antibody at 4°C for 3 h and 25 µL magnetic protein-G beads (Active Motif, Carlsbad, CA, USA) was added for 1 h at 4°C before magnetic capture. Following a series of washes, eluted chromatin was de-crosslinked, proteinase K-treated and ChIP DNA was purified using QIAquick PCR purification kit (Qiagen, Valencia, CA, USA). Eluted ChIP DNA was analysed using quantitative PCR (qPCR). An anti-REST rabbit polyclonal antibody (Millipore Corporation, Bedford, MA, USA) was used to precipitate chromatin. Non-specific binding was assessed by immunoprecipitation with non-immune rabbit IgG and enrichments were normalised to those at the Chrm4 coding region, a locus greater than 10 kb distal to any known RE1 site. RE1 flanking primer sequences were as follows: Bdnf RE1 [Fwd: 5'-GGATTGTCCGAGGTGGTAG-3'; Rev: 5'-CGGAAAAGACGCTTTTAAAGG-3' (these primers are designed to amplify exon 2, present in all Bdnf transcripts)]; Syn1 RE1 (Fwd: 5'-GCACACTCAGAGGGGAACAT-3'; Rev: 5'-CTCTTTCTTTGCCCCGACAG-3'); Chrm4 RE1 (Fwd: 5'-AGGAGACTCGTGCCATCTTC-3'; Rev: 5'-CGAGCATCTACCCTGGCTAA-3').

Immunocytochemistry

Cells were fixed for 10 min in 4% paraformaldehyde, permeabilised in 0.1% Triton X-100 (Sigma) for 5 min then incubated with anti-REST (1 : 200; Upstate Biotechnology, Lake Placid, NY, USA) for 1 h at 20–25°C in phosphate-buffered saline containing 10% normal goat serum. Secondary antibody incubation was performed for 30 min at 20–25°C with Cy5-conjugated rabbit anti-goat IgG (1 : 500; Jackson Labs, CA, USA). Nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI) and coverslips were mounted in Fluoromount-G (Southern Biotech, AL, USA).

BDNF ELISA assays

Cell lysates were prepared in lysis buffer consisting of 10% glycerol, 25 mM Tris HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA and 1 mM EGTA supplemented with 1 : 100 Protease Inhibitor Mixture (Sigma-Aldrich, St Louis, MO, USA). Samples were homogenised, sonicated and centrifuged at maximum speed for 15 min at 4°C. The supernatants were collected and stored at –30°C. Samples were assayed for BDNF using the ImmunoAssay System (Promega) according to manufacturer's instructions.

Nuclear and cytoplasmic western blotting

Cytoplasmic and nuclear protein fractions were extracted using NEPER Nuclear and Cytoplasmic extraction reagents (Thermo scientific, MA, USA) with protease inhibitors (Protease Inhibitor Cocktail; Roche) according to manufacturer's instructions. Protein concentrations were determined using a Bicinchoninic Acid Kit (Sigma) and a Tecan plate reader with Magellan5 software (Tecan, Switzerland). Fifty microgram of nuclear or cytoplasmic protein for each sample was boiled for 5 min in Laemmli sample buffer and run on 6% sodium dodecyl sulphate–polyacrylamide gels. Proteins were transferred to polyvinylidene difluoride membrane using a Mini

Trans-Blot system (Bio-Rad) before blocking in 1× Tris-buffered saline with 0.1% Tween-20 with 5% skimmed-milk for 1 h at 20–25°C. Membranes were then incubated with rabbit anti-REST (Millipore, 1 : 2000), mouse anti-actin (1 : 2000; Abcam, UK) or rabbit anti-RNA Polymerase II (PolII S5, 1 : 2000; Abcam) followed by goat anti-rabbit horseradish peroxidase (1 : 7500; Santa Cruz) or goat anti-mouse horseradish peroxidase (1 : 2000; Dako, Carpinteria, CA, USA) secondary antibodies. Chemiluminescence was performed using Lumi-Light Western Blot Substrate and Lumi-Film (Roche) developed using a CompactX4 X-ray developer. Membranes were stripped between detections using a Re-Blot Plus Kit (Chemicon) according to manufacturer's instructions.

Results

Changes in gene and protein expression following decoy ODN delivery in *Hdh*^{109/109} cells

Hdh^{109/109} cells provide an ideal model system in which to test the effect of a decoy ODN on REST-mediated gene dysregulation since these cells have reduced BDNF mRNA and protein levels compared with *Hdh*^{7/7} cells and show de-repression of *Bdnf* and other REST target genes following infection with a dominant-negative REST (DN : REST) adenovirus (Zuccato *et al.* 2007). Compared with wild-type *Hdh*^{7/7} cells, mutant *Hdh*^{109/109} cells displayed increased nuclear localisation of REST by both immunofluorescence (Fig. 1a) and nuclear and cytoplasmic fraction western blotting (Fig. 1b) as previously reported (Zuccato *et al.* 2003). To target REST function in this system, we designed a decoy ODN (++D) that contained a canonical RE1 sequence, which can be bound by the REST DNA-binding domain. We additionally designed a negative control decoy ODN (--D) carrying a non-canonical RE1 site that cannot be bound by REST (Bruce *et al.* 2004). Both sense and antisense oligonucleotides were synthesised with phosphorothiolate bases at their 5'-ends to protect against exonuclease action. To demonstrate the efficacy and specificity of the REST decoy ODN, we investigated the expression of three REST target genes: *Bdnf*, *Chrm4* and *Syn1* together with β -actin. *Bdnf*, *Chrm4* and *Syn1* have all been shown to be aberrantly repressed by REST in *Hdh*^{109/109} cells (Zuccato *et al.* 2007), whereas β -actin is neither bound nor regulated by REST.

Hdh^{109/109} cells were transfected with the REST decoy ODN (++D) or non-canonical decoy (--D) and expression of *Bdnf*, *Syn1*, *Chrm4* and *Rest* was analysed by qPCR in untransfected *Hdh*^{7/7} and *Hdh*^{109/109} cells and in *Hdh*^{109/109} cells transfected with decoy ODNs 48 h following transfection (Fig. 1c). *Bdnf*, *Syn1* and *Chrm4* all showed significantly reduced expression in *Hdh*^{109/109} compared with control *Hdh*^{7/7} cells. *Hdh*^{109/109} cells transfected with REST decoy ODN showed significant up-regulation of all three REST target genes compared with untransfected cells and compared with cells transfected with --D (Fig. 1c). --D transfected cells showed no significant change in expression

of *Bdnf*, *Syn1* or *Chrm4* compared with untransfected cells (*p*-value = 0.11, 0.12 and 0.05, respectively). REST and actin expression were unaltered in all conditions (Fig. 1c). The observed level of decoy-mediated de-repression of target genes was comparable with that obtained using DN : REST and *Rest* shRNA strategies (Figure S1). In addition, rescue of *Bdnf* gene expression was accompanied by increased *Bdnf* protein levels as measured by ELISA assays (Fig. 1d). Untransfected *Hdh*^{109/109} cells expressed approximately 26% of *Hdh*^{7/7} *Bdnf* levels but following delivery of REST decoy ODN, *Bdnf* protein levels were significantly increased to 65% of wild-type levels (Fig. 1d, ++D) compared with levels following negative decoy (--D) transfection. Our results, therefore, indicate that by attenuating REST binding with modified decoy ODNs, it is possible to de-repress multiple REST target genes and to partially restore *Bdnf* protein levels in HD cells.

REST occupancy of RE1s following decoy ODN delivery

To directly test whether treatment with decoy ODNs decreased occupancy of REST at its RE1 targets in the genome, we carried out ChIP-qPCR analysis using anti-REST IgG and primers that flank RE1 sites of the *Bdnf*, *Syn1* and *Chrm4* genes (Fig. 2). Chromatin from decoy-transfected cells (++D or --D) was collected 48 h post-transfection, similar to gene and protein expression analyses described above earlier, since this time-point showed the highest level of efficacy in rescue of target gene expression (data not shown). REST occupancy was assessed at three REST target gene RE1s: *Bdnf*, *Syn1* and *Chrm4* (Fig. 2). Higher levels of REST occupancy were seen at all three loci in untransfected *Hdh*^{109/109} cells compared with *Hdh*^{7/7} cells, consistent with the higher levels of REST seen in *Hdh*^{109/109} cells. No change in REST occupancy was detected in cells transfected with the negative decoy (Fig. 2, --D). However, following delivery of the *Rest* RE1 decoy ODN, REST occupancy was significantly decreased at all three loci in *Hdh*^{109/109} cells to essentially wild-type (*Hdh*^{7/7}) levels (Fig. 2, ++D). We again performed parallel experiments using DN : REST and *Rest* shRNA for comparison and showed that RE1 decoy ODN was equally as effective at decreasing REST recruitment to these RE1s (Figure S2). Therefore, modified decoy ODNs that contain an RE1 consensus binding site are able to sequester REST protein from target RE1 sites in the genome, resulting in decreased *Rest* occupancy at several target genes, including *Bdnf*.

Changes to histone acetylation of RE1s following decoy delivery

Transcriptionally active or transcriptionally silent chromatin states are accompanied by specific histone amino-terminal modifications conferring distinct epigenetic signatures (for review, see Jenuwein and Allis 2001). Numerous studies have demonstrated that REST is able to maintain low levels of

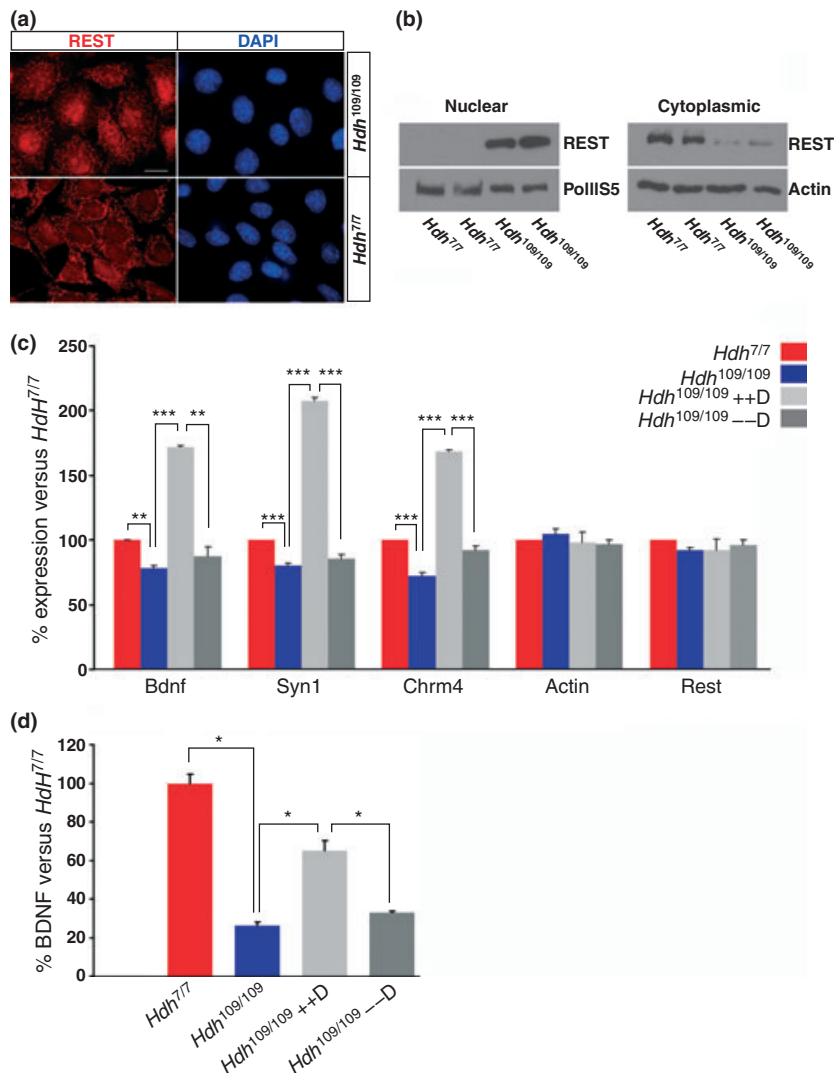


Fig. 1 De-repression of repressor element 1 (RE1) silencing transcription factor (REST) targets and rescue of brain-derived neurotrophic factor (BDNF) protein levels using RE1 decoy oligonucleotides (ODNs). (a) Localisation of REST protein in *Hdh*^{7/7} and *Hdh*^{109/109} cells visualised by immunofluorescence (using anti-REST antibody) and (b) western blot of nuclear and cytoplasmic protein fractions (using anti-REST antibody for both and either anti-RNA polymerase II serine 5 or anti-actin for loading controls respectively). REST is much more highly localised to the nucleus in *Hdh*^{109/109} cells compared with *Hdh*^{7/7} cells. (c) *Bdnf*, *Syn1* and *Chrm4* mRNA levels were measured in *Hdh*^{7/7} and *Hdh*^{109/109} cells following delivery of REST decoy ODN (++D) or negative decoy ODN (--D). All three show reduced

expression in *Hdh*^{109/109} compared with *Hdh*^{7/7} and were increased beyond *Hdh*^{7/7} levels in *Hdh*^{109/109} transfected with ++D compared with untransfected cells and cells transfected with --D. Data are expressed as percentage of expression relative to *Hdh*^{7/7} cell levels and represent the average of three independent experiments. (d) *Bdnf* protein levels were determined by ELISA on lysates from untransfected *Hdh*^{7/7} and *Hdh*^{109/109} cells and *Hdh*^{109/109} cells transfected with ++D or --D. Data are the average of two independent experiments. In (a) scale bar = 20 μ m. For (b) and (c) * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ [using Student's *t*-test in (b) and one-way ANOVA in (c)] and error bars show SDs.

histone H3 lysine 9 acetylation (H3K9ac), an epigenetic marker associated with active chromatin (Dhalluin *et al.* 1999), at the promoter region of target genes via recruitment of histone deacetylase (HDAC) activity (Grimes *et al.* 2000; Roopra *et al.* 2000). To better understand the mechanisms of REST silencing and repression in *Hdh*^{7/7} and *Hdh*^{109/109} cells, we looked at H3K9ac levels around the RE1s in response to

changing REST occupancy using our decoy strategy. Figure 3 shows ChIP-qPCR analysis of H3K9ac levels of REST target genes in untransfected *Hdh*^{7/7} and *Hdh*^{109/109} cells and *Hdh*^{109/109} cells 48 h following delivery of decoy ODNs (++D or --D). *Bdnf* and *Chrm4* showed more than twofold and *Syn1* almost 1.8-fold lower enrichments of H3K9ac in *Hdh*^{109/109} cells compared with *Hdh*^{7/7} cells (Fig. 3, all

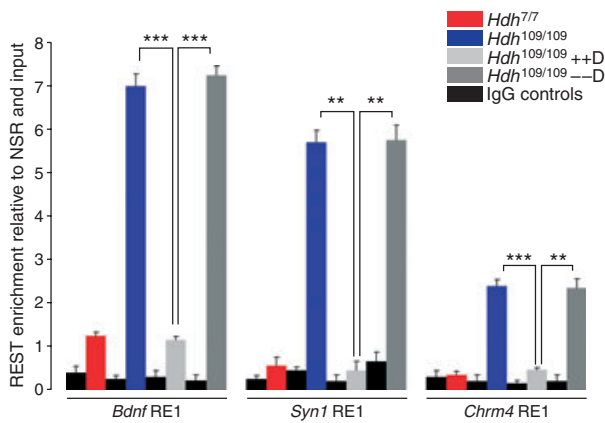


Fig. 2 Repressor element 1 (RE1) silencing transcription factor (REST) occupancy in response to decoy delivery. Chromatin immunoprecipitation (ChIP)-quantitative PCR (qPCR) analysis at three known RE1 sites in untransfected wild-type (*Hdh*^{7/7}) and mutant (*Hdh*^{109/109}) cells and in mutant cells following delivery of canonical RE1-containing decoy (++D) or a non-REST binding RE1-containing decoy (--D). REST occupancy of the *Bdnf*, *Syn1* and *Chrm4* RE1 loci was decreased in *Hdh*^{109/109} cells transfected with ++D compared with untransfected cells and those transfected with --D. Data show REST enrichment relative to both enrichment at a non-specific region that does not contain an RE1 site and to input levels and represent the average of three independent experiments, error bars show SDs, ** $p \leq 0.01$, *** $p \leq 0.001$ (using Student's *t*-test).

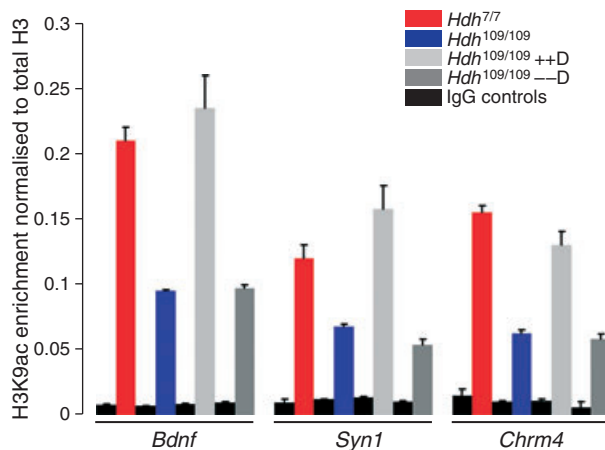


Fig. 3 Effect of decoy delivery on histone H3 acetyl-lysine 9 (H3K9ac) levels at repressor element 1 (RE1) sites. Chromatin immunoprecipitation (ChIP)-quantitative PCR (qPCR) analysis at three known RE1 loci in untransfected wildtype (*Hdh*^{7/7}) and mutant (*Hdh*^{109/109}) cells and in mutant cells after delivery of canonical RE1-containing decoy (++D) or a negative decoy (--D). H3K9ac enrichments in ++D transfected *Hdh*^{109/109} cells at *Bdnf*, *Syn1* and *Chrm4* RE1s were increased to wild-type levels (*Hdh*^{7/7}). H3K9ac enrichment values are shown normalised to total H3 enrichment to account of any variation in nucleosomal density.

$p \leq 0.05$ using Student's *t*-test). We observed a significant increase in H3K9ac enrichments at all three loci, to essentially wild-type levels or greater, following REST decoy ODN delivery (++D) compared with untransfected or with negative decoy ODN (--D) transfected cells (Fig. 3, all $p \leq 0.05$). Our results indicate that REST is able to maintain low levels of H3K9ac around REST binding sites. We also show that regulation of the local chromatin environment is associated with levels of REST occupancy and reduced H3K9ac observed in *Hdh*^{109/109} cells can be restored by the use of modified decoy ODN technology.

RE1-containing decoy ODN interact directly with of REST

To demonstrate a direct interaction between REST and the specific REST RE1 decoy ODN we performed an EMSA with a fluorescent (5'-IRD700) labelled decoy ODN (Fig. 4,

IR-Decoy (++D)	+	+	+	+	+	+	+
Protein	-	-	+	+	+	+	+
Antibody	-	-	-	-	+	+	+
Decoy (--D)	-	-	-	-	-	-	+

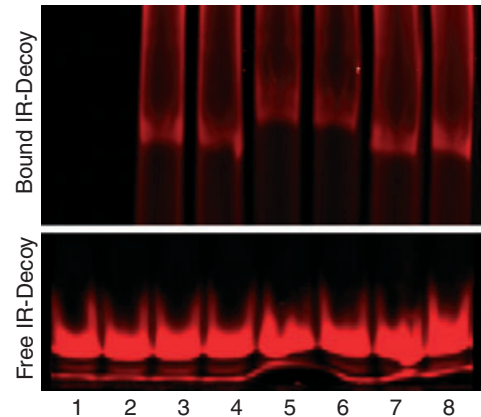


Fig. 4 Decoy oligonucleotides (ODNs) interact directly with repressor element 1 (RE1) silencing transcription factor (REST) protein. The figure shows a fluorescence western blot image with visualisation of an infra-red fluorescence-labelled decoy ODNs containing an RE1-binding site known to bind REST protein (IR-Decoy ++D). The following samples were loaded in duplicate: IR-Decoy ++D alone (lanes 1 and 2); IR-Decoy ++D incubated with HeLa nuclear extract (lanes 3 and 4); IR-Decoy ++D incubated with HeLa nuclear extract and anti-Rest IgG (lanes 5 and 6); HeLa nuclear extract incubated with anti-Rest IgG and excess, non-specific (un-labelled) Decoy ODNs (--D). Free (unbound) IR-Decoy ++D is shown in the bottom panel and alone serves as a negative control (lanes 1 and 2). Incubation of IR-Decoy ++D with HeLa nuclear extract shows an additional band shifted to a higher molecular weight corresponding to a complex of REST protein and IR-Decoy ++D (REST : IR-Decoy). The presence of REST in this complex is confirmed by the super-shifted bands of complexes co-incubated with anti-Rest IgG (lanes 5–6). The REST : IR-Decoy complex is able to form even in the presence of excess quantities of a non-specific, un-labelled decoy containing an RE1 that does not bind to REST (Decoy --D, lanes 7 and 8).

IR-Decoy ++D). The IR-Decoy was incubated with or without HeLa cell nuclear extract and the binding reactions were run on a native polyacrylamide gel. Incubation of the decoy with HeLa cell nuclear extract resulted in a specific band shift corresponding to a protein : decoy (REST : IR-Decoy) interaction (Fig. 4, lanes 3–4). Inclusion of specific anti-REST IgG resulted in a supershift of the protein : decoy complex (Fig. 4, lanes 5–6) that confirmed that complexes shown by the band shifts in lanes 3 and 4 contained REST protein. Moreover, REST : IR-Decoy complexes were not disrupted when binding reactions were co-incubated with excess negative decoy (Fig. 4, lanes 7–8, --D). The EMSA assays confirm the ability and specificity of the modified REST RE1 decoy ODNs to directly interact with REST protein. Therefore, the observed changes to REST target gene expression, protein expression, REST occupancy and the local chromatin environment in *Hdh*^{109/109} cells following introduction of ++D can be attributed to ++D specific sequestration of REST protein.

Discussion

Use of modified decoy ODNs to abrogate the action of transcription factors is a promising approach to rescuing phenotypic defects because of aberrant transcription factor function. In this work, we have developed a decoy strategy to block the activity of REST based on its binding of the conserved 21bp RE1 (Chong *et al.* 1995; Schoenherr *et al.* 1996; Bruce *et al.* 2004; Johnson *et al.* 2006). The *Bdnf* promoter contains an RE1-binding site that has previously been shown to bind to and be regulated by REST both *in vitro* and *in vivo* (Zuccato *et al.* 2001, 2003; Bruce *et al.* 2004) and recent work has highlighted a critical role for REST in HD through its regulation of BDNF (Zuccato *et al.* 2007). Here, EMSA we show the ability of a modified double-stranded ODNs that contains a canonical RE1 site to block binding of REST to endogenous binding sites by sequestration of REST protein and to subsequently rescue levels of BDNF mRNA and protein in *Hdh*^{109/109} cells. Moreover, using ChIP, we show that there is a concomitant decrease in REST occupancy of the RE1 sites in the *Bdnf* promoter and other target genes. In addition, a concomitant local increase in H3K9ac is suggestive of a more open chromatin environment characteristic of activated gene loci. Collectively, these data show that modified decoy ODNs can rescue REST-mediated transcriptional and epigenetic repression in an *in vitro* model of HD.

Decoy ODN can rescue REST target gene expression including BDNF

Widespread transcriptional dysregulation is a central pathogenic mechanism in HD and several transcription factors have been implicated in this disease state (Zuccato *et al.* 2010). Perhaps, the most robust mechanistic link between

transcriptional dysfunction and the pathology of HD is provided by REST (Zuccato and Cattaneo 2007). *In vitro* studies have demonstrated that in normal cells, wild-type Htt sequesters REST in the cytoplasm thereby preventing its binding and repression of target genes (Zuccato *et al.* 2003). However, in the presence of muHtt, this cytoplasmic complex is disrupted and REST migrates to the nucleus where it can bind to and repress its target genes. Although the initial report focussed on the subsequent repression of the BDNF gene (Zuccato *et al.* 2003), we have since demonstrated that REST can bind to and repress numerous genes whose expression is altered in the brain and periphery of HD patients, many of which may also be involved in mediating underlying pathological mechanisms (Zuccato *et al.* 2007; Johnson *et al.* 2008b; Marullo *et al.* 2008). Although many studies, including this study have used cell lines to study the transcriptional effects of mutant Htt, it is important to realise that levels of REST tend to be higher in progenitor lines than differentiated neurons. Nevertheless, several studies have indicated the presence of REST protein in the cytoplasm and nucleus of differentiated neurons (Calderone *et al.* 2003; Kuwabara *et al.* 2004; Sun *et al.* 2005; Spencer *et al.* 2006). Furthermore, one recent study showed that endogenous REST levels were sufficient to repress a RE1-containing BDNF promoter construct in cortical neurons (Hara *et al.* 2009). Accordingly, we propose that abrogation of REST activity offers a potential strategy to rescue aberrantly repressed REST target genes in differentiated neurons and in HD might ultimately restore globally dysregulated neuronal gene transcription.

Using adenoviral delivery of a dominant-negative form of REST, we have previously shown that in addition to *Bdnf*, other REST target genes dysregulated in HD, including *Syn1* and *Chrm4* (encoding synapsin-1 and the M4 muscarinic receptor, respectively), are also de-repressed in parallel with decreased REST occupancy at their RE1 sites (Greenway *et al.* 2007; Zuccato *et al.* 2007; Johnson *et al.* 2008b). Here, we show that transcription of these genes can also be effectively de-repressed using a decoy ODN strategy, thus offering an exciting alternative to the use of viral or plasmid vectors. Further, the efficacy of decoys relies only on having access to the nuclear transcription factor, whereas dominant-negative constructs based on DNA binding domains need to access the chromatin of target genes.

REST acts as an epigenetic regulator in HD

One mechanism by which transcription can be impaired is through altered nucleosome dynamics; in this context, histone methylation and acetylation status is closely linked with transcriptional activity, regulating transcription factor access to promoter regions in DNA (Hake *et al.* 2004; Shogren-Knaak *et al.* 2006). Recent studies have linked HD transcriptional dysregulation to changes in histone modifications (Sadri-Vakili *et al.* 2007; Kim *et al.* 2008), including

hypo-acetylation of histone H3 at promoters of down-regulated genes in R6/2 mice (Sadri-Vakili *et al.* 2007). Moreover, treatment with HDAC inhibitors corrects dysregulated gene expression through modification of histone proteins (Ferrante *et al.* 2003; Sadri-Vakili *et al.* 2007; Thomas *et al.* 2008). This may prove to be of therapeutic value in HD, particularly since several HDAC inhibitors are already approved or being validated in clinical trials (for recent review, see Copeland *et al.* 2010).

REST has been shown to interact with a number of proteins by recruitment of co-repressor platforms via its N- and C-terminal repressor domains (for review, see Ooi and Wood 2007). Many of these proteins are required for REST function via their enzymatic activities that serve to repress or silence gene expression. For example, HDAC1 and HDAC2 are able to repress gene expression by removal of acetyl groups on core histones, including H3K9 (Roopra *et al.* 2000; Ballas *et al.* 2001). Similarly REST recruits H3K4 demethylase activity in the form of lysergic acid diethylamide 1, H3K9 methylase activity via G9a and chromatin re-modelling activity, including brahma-related gene 1 and BRAF35 (Battaglioli *et al.* 2002; Hakimi *et al.* 2002; Roopra *et al.* 2004; Lee *et al.* 2005; Ooi *et al.* 2006). Therefore, REST is able to both silence and repress neuronal genes by creating a chromatin environment bearing a repressive local epigenetic signature (Greenway *et al.* 2007).

Since REST repression in other *in vitro* systems has been shown to be characterised by low levels of H3K9ac (Greenway *et al.* 2007), we used ChIP to assess changes in H3K9ac following delivery of our decoy ODNs into *Hdh*^{109/109} cells. The low levels of H3K9ac observed around the RE1 sites of REST target genes in *Hdh*^{109/109} cells compared with wild-type *Hdh*^{7/7} cells is in keeping with the parallel decreases observed in gene expression and the reciprocally increased occupancy of REST at these sites. ++D decoy-transfected cells, however, showed significantly increased enrichment of H3K9ac at these loci to essentially wild-type levels. These data suggest that REST may have a dual function in target gene repression, including *Bdnf*, acting both as a transcriptional repressor and epigenetic modulator. This likely occurs through direct binding to its RE1 site and subsequent modification of the surrounding chromatin environment. In this regard, we propose that REST may be considered as much an epigenetic regulator as a transcriptional repressor during the pathological state of HD.

Potential of modified decoy ODN technology in HD

Over 3000 REST binding sites have been identified in the human and murine genomes, and many target genes encode for proteins that are fundamental for neuronal development and differentiation (Bruce *et al.* 2004; Johnson *et al.* 2006). REST is the only known transcription factor able to bind the highly conserved RE1 sequence. Owing to the specificity of this binding and the long 21 bp length of the canonical RE1

sequence, modulation of aberrant REST function associated with HD represents an ideal model for the application of decoy ODN strategies. One of the main limitations of decoy technology has been the rapid degradation of unmodified ODNs by nuclease digestion (Gao *et al.* 1992; Brown *et al.* 1994). Different strategies have evolved to render the decoys nuclease-resistant, such as the use of locked nucleic acids (Crinelli *et al.* 2002) or addition of phosphorothiolated nucleotides (Yang *et al.* 2002). We chose the latter approach and used a phosphorothiolated decoy ODN based on a canonical RE1 site (REST decoy) as defined by previous bioinformatic and ChIP studies (Bruce *et al.* 2004; Johnson *et al.* 2006). Results presented here demonstrate that a phosphorothiolated decoy is sufficiently stable and specific to enable efficient blockade of REST activity. In addition, our results suggest that decoy ODNs are as effective at attenuating REST activity as other previously employed strategies, such as adenoviral delivery of DN : REST or transfection of Rest shRNAs (Greenway *et al.* 2007; Zuccato *et al.* 2007 and data not shown). They also avoid the need for large DNA constructs and viral delivery systems and they side-step the issue of potential off-target effects encountered using RNAi technology. Moreover, their ability to directly interfere with transcription factor binding can be directly inferred by using labelled ODNs to serve as probes in EMSAs (Hara *et al.* 2003; Kubo *et al.* 2003). With a fluorescence-labelled decoy ODN carrying the canonical RE1 sequence, we were able to directly show the interaction between REST and the decoy and we confirmed the presence of REST in this complex through the use of a specific anti-REST antibody to supershift the protein/DNA complex.

Widespread gene dysregulation is a hallmark of HD and in some cases precedes many of the well-known physical symptoms of the disease (for recent review, see Buckley *et al.* 2010). The functional products of many of these dysregulated genes have provided new insights into possible mechanisms underlying HD pathology, including the well-known role of BDNF and concomitant loss of medium spiny neurons (reviewed in Zuccato and Cattaneo 2007). As well as transcriptional dysregulation, it is becoming increasingly clear that epigenetic disruption occurs in HD and, indeed, targeting HDAC activity has become a major platform for HD drug development (Ryu *et al.* 2006; Kazantsev and Hersch 2007; Kazantsev and Thompson 2008). Many REST functions are attributed to its recruitment of HDAC activity and it is likely that the causative role of REST in HD occurs through an inextricable linkage of transcriptional and epigenetic dysregulation (Buckley *et al.* 2010). The use of a modified decoy strategy presented here that specifically targets REST function provides a novel and effective means by which it is possible to show effective rescue of both transcriptional and epigenetic effects of REST in an *in vitro* HD model; this included restoration of *Bdnf* gene and protein expression and H3K9ac levels at its promoter. Modified

decoys thus offer an attractive alternative to plasmid- or viral-based vectors for interfering with target protein function and provide an ideal strategy to investigate the contribution of REST to HD pathology in well-established mouse models. Perhaps, more intriguingly, since modified ODNs targeting transcription factor function have been shown to be of potential therapeutic value in other disorders (Kubo *et al.* 2003; De Stefano *et al.* 2010), they may be useful in future clinical intervention for early-stage HD. The work presented here presents a proof-of-concept that our decoy strategy is capable of rescuing REST-mediated repression of neuronal gene expression a cell line model of HD. Nevertheless, these findings need to be taken forward and the effectiveness of decoys needs to be tested in neuronal cells, where endogenous levels of REST are lower than those found in Hdh cells. Future studies will address this by using *in vivo* models of HD.

Acknowledgements

This work is funded by a fellowship from Fondazione Cenci Bolognietti (to CS), the Wellcome Trust (to NJB), the Huntington's Disease Society of America (H.D.S.A., New York) to EC and the Cure HD Initiative (CHDI) Foundation (New York) to EC and NJB. The authors have no competing interests to declare.

Supporting information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Supplementary Materials and methods.

Figure S1. Reduced REST target gene repression in *Hdh*^{109/109} cells using dominant-negative REST or Rest shRNA.

Figure S2. Reduced REST occupancy of RE1 sites in *Hdh*^{109/109} cells using dominant-negative REST or Rest shRNA.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organised for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

References

- Abrajano J. J., Qureshi I. A., Gokhan S., Zheng D., Bergman A. and Mehler M. F. (2009a) Differential deployment of REST and CoREST promotes glial subtype specification and oligodendrocyte lineage maturation. *PLoS ONE* **4**, e7665.
- Abrajano J. J., Qureshi I. A., Gokhan S., Zheng D., Bergman A. and Mehler M. F. (2009b) REST and CoREST modulate neuronal subtype specification, maturation and maintenance. *PLoS ONE* **4**, e7936.
- Aharoni-Simon M., Reifsnider R. and Tirosh O. (2006) ROS-production-mediated activation of AP-1 but not NFκB inhibits glutamate-induced HT4 neuronal cell death. *Antioxid. Redox Signal.* **8**, 1339–1349.
- Altar C. A., Cai N., Bliven T., Juhasz M., Conner J. M., Acheson A. L., Lindsay R. M. and Wiegand S. J. (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. *Nature* **389**, 856–860.
- Bae B. I., Xu H. and Igarashi S. *et al.* (2005) p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* **47**, 29–41.
- Ballas N., Battaglioli E. and Atouf F. *et al.* (2001) Regulation of neuronal traits by a novel transcriptional complex. *Neuron* **31**, 353–365.
- Bates G. P. (2005) History of genetic disease: the molecular genetics of Huntington disease - a history. *Nat. Rev. Genet.* **6**, 766–773.
- Battaglioli E., Andres M. E., Rose D. W., Chenoweth J. G., Rosenfeld M. G., Anderson M. E. and Mandel G. (2002) REST repression of neuronal genes requires components of the hSWI.SNF complex. *J. Biol. Chem.* **277**, 41038–41045.
- Bithell A., Johnson R. and Buckley N. J. (2009) Transcriptional dysregulation of coding and non-coding genes in cellular models of Huntington's disease. *Biochem. Soc. Trans.* **37**, 1270–1275.
- Brown D. A., Kang S. H., Gryaznov S. M., DeDionisio L., Heidenreich O., Sullivan S., Xu X. and Nerenberg M. I. (1994) Effect of phosphorothioate modification of oligodeoxynucleotides on specific protein binding. *J. Biol. Chem.* **269**, 26801–26805.
- Bruce A. W., Donaldson I. J., Wood I. C., Yerbury S. A., Sadowski M. I., Chapman M., Gottgens B. and Buckley N. J. (2004) Genome-wide analysis of repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF) target genes. *Proc. Natl Acad. Sci. USA* **101**, 10458–10463.
- Buckley N. J., Johnson R., Zuccato C., Bithell A. and Cattaneo E. (2010) The role of REST in transcriptional and epigenetic dysregulation in Huntington's disease. *Neurobiol. Dis.* **39**, 28–39.
- Calderone A., Jover T. and Noh K. M. *et al.* (2003) Ischemic insults derepress the gene silencer REST in neurons destined to die. *J. Neurosci.* **23**, 2112–2121.
- Canals J. M., Pineda J. R., Torres-Peraza J. F., Bosch M., Martin-Ibanez R., Munoz M. T., Mengod G., Ernors P. and Alberch J. (2004) Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. *J. Neurosci.* **24**, 7727–7739.
- Cattaneo E., Zuccato C. and Tartari M. (2005) Normal huntingtin function: an alternative approach to Huntington's disease. *Nat. Rev. Neurosci.* **6**, 919–930.
- Chae Y. M., Park K. K., Lee I. K., Kim J. K., Kim C. H. and Chang Y. C. (2006) Ring-Sp1 decoy oligonucleotide effectively suppresses extracellular matrix gene expression and fibrosis of rat kidney induced by unilateral ureteral obstruction. *Gene Ther.* **13**, 430–439.
- Chong J. A., Tapia-Ramirez J. and Kim S. *et al.* (1995) REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell* **80**, 949–957.
- Copeland R. A., Olhava E. J. and Scott M. P. (2010) Targeting epigenetic enzymes for drug discovery. *Curr. Opin. Chem. Biol.* **14**, 505–510.
- Crinelli R., Bianchi M., Gentilini L. and Magnani M. (2002) Design and characterization of decoy oligonucleotides containing locked nucleic acids. *Nucleic Acids Res.* **30**, 2435–2443.
- Dash P. K., Orsi S. A. and Moore A. N. (2005) Sequestration of serum response factor in the hippocampus impairs long-term spatial memory. *J. Neurochem.* **93**, 269–278.
- De Stefano D., De Rosa G. and Carnuccio R. (2010) NFκB decoy oligonucleotides. *Curr. Opin. Mol. Ther.* **12**, 203–213.
- Dhalluin C., Carlson J. E., Zeng L., He C., Aggarwal A. K. and Zhou M. M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399**, 491–496.
- Dunah A. W., Jeong H. and Griffin A. *et al.* (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* **296**, 2238–2243.

- Ferrante R. J., Kubilus J. K. and Lee J. *et al.* (2003) Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *J. Neurosci.* **23**, 9418–9427.
- Gao W. Y., Han F. S., Storm C., Egan W. and Cheng Y. C. (1992) Phosphorothioate oligonucleotides are inhibitors of human DNA polymerases and RNase H: implications for antisense technology. *Mol. Pharmacol.* **41**, 223–229.
- Garriga-Canut M., Schoenike B. and Qazi R. *et al.* (2006) 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat. Neurosci.* **9**, 1382–1387.
- Greenway D. J., Street M., Jeffries A. and Buckley N. J. (2007) RE1 Silencing transcription factor maintains a repressive chromatin environment in embryonic hippocampal neural stem cells. *Stem Cells* **25**, 354–363.
- Griesenbach U., Cassady R. L., Cain R. J., duBois R. M., Geddes D. M. and Alton E. W. (2002) Cytoplasmic deposition of NFkappaB decoy oligonucleotides is insufficient to inhibit bleomycin-induced pulmonary inflammation. *Gene Ther.* **9**, 1109–1115.
- Grimes J. A., Nielsen S. J. and Battaglioli E. *et al.* (2000) The co-repressor mSin3A is a functional component of the REST-CoREST repressor complex. *J. Biol. Chem.* **275**, 9461–9467.
- Hake S. B., Xiao A. and Allis C. D. (2004) Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br. J. Cancer* **90**, 761–769.
- Hakimi M. A., Bochar D. A., Chenoweth J., Lane W. S., Mandel G. and Shiekhatter R. (2002) A core-BRAF35 complex containing histone deacetylase mediates repression of neuronal-specific genes. *Proc. Natl Acad. Sci. USA* **99**, 7420–7425.
- Hara T., Hamada J., Yano S., Morioka M., Kai Y. and Ushio Y. (2003) CREB is required for acquisition of ischemic tolerance in gerbil hippocampal CA1 region. *J. Neurochem.* **86**, 805–814.
- Hara D., Fukuchi M., Miyashita T., Tabuchi A., Takasaki I., Naruse Y., Mori N., Kondo T. and Tsuda M. (2009) Remote control of activity-dependent BDNF gene promoter-I transcription mediated by REST/NRSF. *Biochem. Biophys. Res. Commun.* **384**, 506–511.
- Huang C. C., Faber P. W., Persichetti F., Mittal V., Vonsattel J. P., MacDonald M. E. and Gusella J. F. (1998) Amyloid formation by mutant huntingtin: threshold, progressivity and recruitment of normal polyglutamine proteins. *Somat. Cell Mol. Genet.* **24**, 217–233.
- Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* **72**, 971–983.
- Jenuwein T. and Allis C. D. (2001) Translating the histone code. *Science* **293**, 1074–1080.
- Johnson R. and Buckley N. J. (2009) Gene Dysregulation in Huntington's Disease: REST, MicroRNAs and Beyond. *Neuromol. Med.* **11**, 183–199.
- Johnson R., Gamblin R. J., Ooi L., Bruce A. W., Donaldson I. J., Westhead D. R., Wood I. C., Jackson R. M. and Buckley N. J. (2006) Identification of the REST regulon reveals extensive transposable element-mediated binding site duplication. *Nucleic Acids Res.* **34**, 3862–3877.
- Johnson D. S., Mortazavi A., Myers R. M. and Wold B. (2007) Genome-wide mapping of in vivo protein-DNA interactions. *Science* **316**, 1497–1502.
- Johnson R., Teh C. H. and Kunarso G. *et al.* (2008a) REST regulates distinct transcriptional networks in embryonic and neural stem cells. *PLoS Biol.* **6**, e256.
- Johnson R., Zuccato C., Belyaev N. D., Guest D. J., Cattaneo E. and Buckley N. J. (2008b) A microRNA-based gene dysregulation pathway in Huntington's disease. *Neurobiol. Dis.* **29**, 438–445.
- Kazantsev A. G. and Hersch S. M. (2007) Drug targeting of dysregulated transcription in Huntington's disease. *Prog. Neurobiol.* **83**, 249–259.
- Kazantsev A. G. and Thompson L. M. (2008) Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat. Rev. Drug Discov.* **7**, 854–868.
- Kim M. O., Chawla P., Overland R. P., Xia E., Sadri-Vakili G. and Cha J. H. (2008) Altered histone monoubiquitylation mediated by mutant huntingtin induces transcriptional dysregulation. *J. Neurosci.* **28**, 3947–3957.
- Kraner S. D., Chong J. A., Tsay H. J. and Mandel G. (1992) Silencing the type II sodium channel gene: a model for neural-specific gene regulation. *Neuron* **9**, 37–44.
- Kubo T., Kinjyo N., Ikezawa A., Kambe T. and Fukumori R. (2003) Sp1 decoy oligodeoxynucleotide decreases angiotensin receptor expression and blood pressure in spontaneously hypertensive rats. *Brain Res.* **992**, 1–8.
- Kuwabara T., Hsieh J., Nakashima K., Taira K. and Gage F. H. (2004) A small modulatory dsRNA specifies the fate of adult neural stem cells. *Cell* **116**, 779–793.
- Lee I. K., Ahn J. D., Kim H. S., Park J. Y. and Lee K. U. (2003) Advantages of the circular dumbbell decoy in gene therapy and studies of gene regulation. *Curr. Drug Targets* **4**, 619–623.
- Lee M. G., Wynder C., Cooch N. and Shiekhatter R. (2005) An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* **437**, 432–435.
- Marullo M., Valenza M., Mariotti C., Di Donato S., Cattaneo E. and Zuccato C. (2008) Analysis of the Repressor Element-1 Silencing Transcription Factor/Neuron-Restrictive Silencer Factor Occupancy of Non-Neuronal Genes in Peripheral Lymphocytes from Patients with Huntington's Disease. *Brain Pathol.* **20**, 96–105.
- Meller R., Minami M., Cameron J. A., Impey S., Chen D., Lan J. Q., Henshall D. C. and Simon R. P. (2005) CREB-mediated Bcl-2 protein expression after ischemic preconditioning. *J. Cereb. Blood Flow Metab.* **25**, 234–246.
- Mori N., Schoenherr C., Vandenberg D. J. and Anderson D. J. (1992) A common silencer element in the SCG10 and type II Na⁺ channel genes binds a factor present in nonneuronal cells but not in neuronal cells. *Neuron* **9**, 45–54.
- Nucifora Jr. F. C., Sasaki M. and Peters M. F. *et al.* (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291**, 2423–2428.
- Ooi L. and Wood I. C. (2007) Chromatin crosstalk in development and disease: lessons from REST. *Nat. Rev. Genet.* **8**, 544–554.
- Ooi L., Belyaev N. D., Miyake K., Wood I. C. and Buckley N. J. (2006) BRG1 chromatin remodeling activity is required for efficient chromatin binding by repressor element 1-silencing transcription factor (REST) and facilitates REST-mediated repression. *J. Biol. Chem.* **281**, 38974–38980.
- Osako M. K., Tomita N. and Nakagami H. *et al.* (2007) Increase in nuclease resistance and incorporation of NF-kappaB decoy oligodeoxynucleotides by modification of the 3'-terminus. *J. Gene Med.* **9**, 812–819.
- Palm K., Belluardo N., Metsis M. and Timmusk T. (1998) Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. *J. Neurosci.* **18**, 1280–1296.
- Pfaffl M. W., Georgieva T. M., Georgiev I. P., Ontsouka E., Hageleit M. and Blum J. W. (2002) Real-time RT-PCR quantification of insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. *Domest. Anim. Endocrinol.* **22**, 91–102.
- Roopra A., Sharling L., Wood I. C., Briggs T., Bachfischer U., Paquette A. J. and Buckley N. J. (2000) Transcriptional repression by

- neuron-restrictive silencer factor is mediated via the Sin3-histone deacetylase complex. *Mol. Cell. Biol.* **20**, 2147–2157.
- Roopra A., Qazi R., Schoenike B., Daley T. J. and Morrison J. F. (2004) Localized domains of G9a-mediated histone methylation are required for silencing of neuronal genes. *Mol. Cell* **14**, 727–738.
- Rubinsztein D. C. and Carmichael J. (2003) Huntington's disease: molecular basis of neurodegeneration. *Expert Rev. Mol. Med.* **5**, 1–21.
- Ryu H., Lee J., Hagerty S. W., Soh B. Y., McAlpin S. E., Cormier K. A., Smith K. M. and Ferrante R. J. (2006) ESET/SETDB1 gene expression and histone H3 (K9) trimethylation in Huntington's disease. *Proc. Natl Acad. Sci. USA* **103**, 19176–19181.
- Sadri-Vakili G., Bouzou B. and Benn C. L. *et al.* (2007) Histones associated with downregulated genes are hypo-acetylated in Huntington's disease models. *Hum. Mol. Genet.* **16**, 1293–1306.
- Schoenherr C. J. and Anderson D. J. (1995) The neuron-restrictive silencer factor (NRSF): a coordinate repressor of multiple neuron-specific genes. *Science* **267**, 1360–1363.
- Schoenherr C. J., Paquette A. J. and Anderson D. J. (1996) Identification of potential target genes for the neuron-restrictive silencer factor. *Proc. Natl Acad. Sci. USA* **93**, 9881–9886.
- Shogren-Knaak M., Ishii H., Sun J. M., Pazin M. J., Davie J. R. and Peterson C. L. (2006) Histone H4–K16 acetylation controls chromatin structure and protein interactions. *Science* **311**, 844–847.
- Spencer E. M., Chandler K. E. and Haddley K. *et al.* (2006) Regulation and role of REST and REST4 variants in modulation of gene expression in vivo and in vitro in epilepsy models. *Neurobiol. Dis.* **24**, 41–52.
- Stadlbauer T. H., Wagner A. H., Holschermann H., Fiedel S., Fingerhuth H., Tillmanns H., Bohle R. M. and Hecker M. (2008) AP-1 and STAT-1 decoy oligodeoxynucleotides attenuate transplant vasculopathy in rat cardiac allografts. *Cardiovasc. Res.* **79**, 698–705.
- Steffan J. S., Kazantsev A. and Spasic-Boskovic O. *et al.* (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA* **97**, 6763–6768.
- Steiger J. L., Bandyopadhyay S., Farb D. H. and Russek S. J. (2004) cAMP response element-binding protein, activating transcription factor-4, and upstream stimulatory factor differentially control hippocampal GABABR1a and GABABR1b subunit gene expression through alternative promoters. *J. Neurosci.* **24**, 6115–6126.
- Sun Y. M., Greenway D. J., Johnson R., Street M., Belyaev N. D., Deuchars J., Bee T., Wilde S. and Buckley N. J. (2005) Distinct Profiles of REST Interactions with Its Target Genes at Different Stages of Neuronal Development. *Mol. Biol. Cell* **16**, 5630–5638.
- Thomas E. A., Coppola G. and Desplats P. A. *et al.* (2008) The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. *Proc. Natl Acad. Sci. USA* **105**, 15564–15569.
- Trettel F., Rigamonti D., Hilditch-Maguire P., Wheeler V. C., Sharp A. H., Persichetti F., Cattaneo E. and MacDonald M. E. (2000) Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. *Hum. Mol. Genet.* **9**, 2799–2809.
- Yang Y., Tesmer V. M. and Bina M. (2002) Regulation of HIV-1 transcription in activated monocyte macrophages. *Virology* **299**, 256–265.
- Yao Y., Kubota T., Sato K., Takeuchi H., Kitai R. and Matsukawa S. (2005) Interferons upregulate thymidine phosphorylase expression via JAK-STAT-dependent transcriptional activation and mRNA stabilization in human glioblastoma cells. *J. Neurooncol.* **72**, 217–223.
- Ye S. M. and Johnson R. W. (2001) Regulation of interleukin-6 gene expression in brain of aged mice by nuclear factor kappaB. *J. Neuroimmunol.* **117**, 87–96.
- Zala D., Benchoua A., Brouillet E., Perrin V., Gaillard M. C., Zurn A. D., Aebischer P. and Deglon N. (2005) Progressive and selective striatal degeneration in primary neuronal cultures using lentiviral vector coding for a mutant huntingtin fragment. *Neurobiol. Dis.* **20**, 785–798.
- Zuccato C. and Cattaneo E. (2007) Role of brain-derived neurotrophic factor in Huntington's disease. *Prog. Neurobiol.* **81**, 294–330.
- Zuccato C. and Cattaneo E. (2009) Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat. Rev. Neurol.* **5**, 311–322.
- Zuccato C., Ciammola A. and Rigamonti D. *et al.* (2001) Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. *Science* **293**, 493–498.
- Zuccato C., Tartari M. and Crotti A. *et al.* (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat. Genet.* **35**, 76–83.
- Zuccato C., Belyaev N. and Conforti P. *et al.* (2007) Widespread disruption of repressor element-1 silencing transcription factor/neuron-restrictive silencer factor occupancy at its target genes in Huntington's disease. *J. Neurosci.* **27**, 6972–6983.
- Zuccato C., Marullo M., Conforti P., MacDonald M. E., Tartari M. and Cattaneo E. (2008) Systematic assessment of BDNF and its receptor levels in human cortices affected by Huntington's disease. *Brain Pathol.* **18**, 225–238.
- Zuccato C., Valenza M. and Cattaneo E. (2010) Molecular mechanisms and potential therapeutic targets in Huntington's disease. *Physiol. Rev.* **90**, 905–981.